Volume 112 Number 3 September 1996

The Journal of
THORACIC AND CARDIOVASCULAR SURGERY

CARDIAC AND PULMONARY REPLACEMENT

A NEWLY DEVELOPED SOLUTION ENHANCES THIRTY-HOUR PRESERVATION IN A CANINE LUNG TRANSPLANTATION MODEL

Chun Jiang Liu, MD
Mitsuhiro Ueda, MD
Shinji Kosaka, MD
Toshiki Hirata, MD
Hiroyasu Yokomise, MD
Kenji Inui, MD
Shigeki Hitomi, MD
Hiromi Wada, MD

Ischemia and reperfusion cause the production of oxygen free radicals. These damage grafts or disrupt normal vascular homeostatic mechanisms, with a parallel reduction in endothelial nitric oxide and adenosine 3′,5′-cyclic monophosphate levels. We hypothesized that lung preservation failure may be related to these events. To improve lung preservation, we prepared a new ET-Kyoto solution, which contains *N*-acetylcysteine (a radical scavenger), nitroglycerin (to elevate the nitric oxide level), and dibutyryl adenosine 3′,5′-cyclic monophosphate (to elevate the adenosine 3′,5′-cyclic monophosphate level) and examined its efficacy in a canine single-lung transplantation model. Lungs were flushed with new ET-Kyoto solution (group I, $n = 9$), basal ET-Kyoto solution (group II, $n = 6$), basal ET-Kyoto solution plus ethanol and propylene glycol (solvents of nitroglycerin; group III, $n = 6$), or low-potassium dextran glucose solution (group IV, $n = 6$), and stored at 4° C for 30 hours. After left single-lung transplantation, the right main bronchus and right pulmonary artery were ligated and the functions of the transplanted lung were assessed for 6 hours. Arterial oxygen tension was significantly higher in group I than in groups II, III, and IV ($p < 0.05$). Peak inspiratory pressure and wet-to-dry lung weight ratio were significantly lower in group I than in groups II and IV ($p < 0.01$). Histologic and ultrastructural studies showed better preservation in group I than in groups II, III, and IV. We conclude that the new ET-Kyoto solution provides enhanced 30-hour lung preservation.
(J Thorac Cardiovasc Surg 1996;112:569-76)

From the Department of Thoracic Surgery, Chest Disease Research Institute, Kyoto University, Kyoto, Japan.
Received for publication Oct. 24, 1995; revisions requested Jan. 1, 1996; revisions received Jan. 31, 1996; accepted for publication Feb. 5, 1995.

Address for reprints: Hiromi Wada, MD, Department of Thoracic Surgery, Chest Disease Research Institute, Kyoto University, 53 Shogoin Sakyo-ku, Kyoto 606, Japan.

0022-5223/96 $5.00 + 0 **12/1/72901**

The shortage of suitable donor lungs still limits the widespread application of lung transplantation among patients with end-stage lung diseases.[1] Current commercial preservation solutions used in clinical lung transplantation have a maximum safe ischemic time of 10 hours.[2, 3] Because the lung has a delicate alveolar–capillary membrane network, it is important to maintain the integrity of the pulmonary vascular endothelium during preservation and transplantation. In our previous study, we demonstrated by scanning electron microscopy (SEM) and transmission electron microscopy a statistically significant correlation between the functions of the transplanted lung and the structures of the vascular endothelial cells.[4] In a series of studies, ischemia and reperfusion were shown to produce many oxygen free radicals, which caused pulmonary vascular endothelial cell damage.[5, 6] Furthermore, ischemia and reperfusion reduce endothelial nitric oxide (NO) and adenosine 3′,5′-cyclic monophosphate (cAMP) levels,[7, 8] both of which play critical roles in maintaining the vascular endothelial barrier function and in preventing the adherence to vascular endothelial cells of neutrophils and platelets.[8-12] Many reports have shown that oxygen–free radical scavengers, such as *N*-acetylcysteine, protect pulmonary endothelial cells from ischemia-reperfusion injury. Agents such as nitroglycerin or dibutyryl cAMP (db-cAMP), which elevate NO or cAMP levels, maintain graft vascular homeostasis during ischemia and reperfusion.[5, 6, 13-17] We hypothesized that adequate supplementation of a preservation solution with these agents might enhance lung preservation for transplantation. We prepared a new ET-Kyoto solution (new ET-K) in which *N*-acetylcysteine, nitroglycerin, and db-cAMP are added to the previously reported ET-Kyoto solution (ET-K),[18, 19] and examined its efficacy for 30-hour preservation of canine lungs prepared for transplantation.

Materials and methods

Solutions. The compositions of the solutions are shown in Table I. *N*-acetylcysteine was supplied by Eisai Co., Ltd. (Tokyo, Japan), nitroglycerin was purchased from the Green Cross Corporation (Osaka, Japan), db-cAMP was purchased from Daiiti Pharmaceutical Co., Ltd. (Tokyo, Japan), ethanol was purchased from Ueno Kagaku Inc. (Kyoto, Japan), and propylene glycol was purchased from Nacalai Tesque Inc. (Kyoto, Japan). ET-K (osmolarity 366 mOsm/L) was prepared by us as described elsewhere.[18, 19] New ET-K was prepared just before the donor lung was flushed. Ethanol and propylene glycol were included new ET-K as solvents of nitroglycerin, and the osmolarity of new ET-K was 598 mOsm/L. To determine whether these two solvents and the osmolarity of new ET-K would have any effect on lung preservation, we prepared ET-K with added ethanol and propylene glycol (ET-KA), the osmolarity of which was 583 mOsm/L. The low-potassium dextran glucose solution (LPDG) used in this study was prepared as described in previous reports.[20, 21] The compositions of these solutions were analyzed before the experiments.

Experimental groups. Twenty-seven pairs of size-matched adult mongrel dogs weighing 7.7 to 15.7 kg were randomly assigned to four experimental groups. In group I ($n = 9$), donor lungs were flushed and stored with new ET-K, in group II ($n = 6$) donor lungs were flushed and stored with ET-K, in group III ($n = 6$)donor lungs were flushed and stored with ET-KA, and in group IV ($n = 6$) donor lungs were flushed and stored with LPDG.

Anesthesia. Induction and maintenance of anesthesia were conducted as described previously.[18, 19, 22] Both the donor animals and the recipient animals were ventilated with an inspired oxygen fraction of 0.5 at a tidal volume of 20 ml/kg, a respiratory rate of 15 breaths/min, and a positive end-expiratory pressure of 5 cm H_2O. During operative procedures, nitrous oxide with 0.5% to 1.0% halothane was used.

Donor procedure. A right femoral venous catheter (Swan-Ganz catheter; Baxter Healthcare Corp., Edwards Div., Irvine, Calif.) and a right femoral arterial catheter were introduced. Arterial blood gases, peak inspiratory pressure, systemic hemodynamics (cardiac output [CO], systemic blood pressure), and pulmonary hemodynamics (pulmonary arterial pressure [PAP], pulmonary capillary wedge pressure [PCWP]) were measured. Pulmonary vascular resistance (PVR) was calculated as follows: $PVR = [(PAP - PCWP)/CO] \times (80\ dyne \cdot sec \cdot cm^{-5})$. After median sternotomy, the azygos vein was transected and the superior and inferior venae cavae, aorta, and pulmonary artery were encircled. After systemic heparinization (200 U/kg), the main pulmonary artery was cannulated with a 3-0 Prolene purse-string suture (Ethicon, Inc., Somerville, N.J.). A large bolus (25 μg/kg) of prostaglandin E_1 was injected into the right ventricular outflow tract. When the systemic systolic pressure declined by at least 40%, the superior and inferior venae cavae and the aorta were transected and the proximal pulmonary artery was ligated. The left atrial appendage was amputated, and the lungs were inflated to a maximum inspiratory pressure until all atelectasis had been eliminated. The pulmonary artery was flushed by gravity from a height of 50 cm with 70 ml/kg cold (4° C) perfusate (new ET-K, ET-K, ET-KA, or LPDG). Ventilation of the lungs was continued during pulmonary artery flushing, and the duration of flushing was recorded. The defect at the cannula insertion site in the pulmonary artery was closed after removal of the cannula, and the left atrial appendage was ligated. At an endotracheal pressure of 20 cm H_2O, the trachea was clamped and the heart-lung block was excised with minimal handling of both lungs. The block was then placed in a sterile plastic bag containing 1000 ml of the corresponding cold solution with 400,000 U penicillin (Meiji Seika,

Table I. *Composition of preservation solutions*

	New ET-K	*ET-K*	*ET-KA*	*LPDG*
Sodium (mmol/L)	107	100	99	165
Potassium (mmol/L)	42	44	43	4
Magnesium (mmol/L)	—	—	—	2
Chloride (mmol/L)	—	—	—	101
Sulfate (mmol/L)	—	—	—	2
Phosphate (mmol/L)	24	25	25	34
Gluconate (mmol/L)	97	100	99	—
Glucose (mmol/L)	—	—	—	56
Trehalose (mmol/L)	117	120	119	—
Dextran 40 (gm/L)	—	—	—	20
Hydroxyethyl starch (gm/L)	29	30	30	—
N-acetylcysteine (mmol/L)	10	—	—	—
db-cAMP (mmol/L)	2	—	—	—
Nitroglycerin (mmol/L)	0.44	—	—	—
Ethanol* (mmol/L)	96	—	96	—
Propyleneglycol* (mmol/L)	76	—	76	—
pH	7.4	7.4	7.4	7.4
Osmolarity (mOsm/L)	598	366	583	335

*Solvent of nitroglycerin.

Ltd., Tokyo, Japan) and stored at 4° C for 30 hours. After cold preservation for 30 hours, parts of the apical, medial, and lateral basal segments of the right donor lung were excised for SEM examination by two histopathologists who were unaware of the preservation solutions used.

Recipient procedure. Recipients were anesthetized, and Swan-Ganz and arterial catheters were introduced as in the donor procedure. None of the recipients received heparin. Arterial blood gases, peak inspiratory pressure, and systemic and pulmonary hemodynamic data (as listed in donor procedure) were recorded. Immediately after pneumonectomy, parts of the anterior and posterior basal segments of the resected native left lung were excised and air-dried at 70° C for 7 days, and the wet-to-dry lung weight ratio was calculated. Left single-lung transplantation was performed as described elsewhere.[18, 19, 22] Anastomoses were performed in the following order: left atrium, left main bronchus, and left pulmonary artery. After transplantation, the right main bronchus and the right pulmonary artery were ligated 30 minutes after reperfusion, after the tidal volume had been reduced to two thirds. Arterial blood gases, peak inspiratory pressure, PAP, and systemic blood pressure were recorded 1, 2, 3, 4, 5, and 6 hours after reperfusion. CO and left atrial pressure (LAP) were recorded 6 hours after reperfusion. PVR was calculated as follows: $PVR = [(PAP - LAP)/CO] \times 80$ dyne · sec · cm^{-5}. After the final assessments, the animals were killed. Parts of the anterior and posterior basal segments of the transplanted lung were excised and air-dried at 70° C for 7 days, and the wet-to-dry lung weight ratio was calculated. One piece from the apical posterior segment and another piece from the lateral basal segment of the transplanted left lung were excised for histologic findings by two histopathologists who were unaware of the preservation solutions used.

Table II. *Donor lung data*

	Group I (n = 9)	*Group II (n = 6)*	*Group III (n = 6)*	*Group IV (n = 6)*
Flushing time (sec)*	71 ± 6	98 ± 16	85 ± 10	64 ± 8
Cold ischemic time (min)*	1802 ± 9	1803 ± 8	1799 ± 10	1808 ± 3
Warm ischemic time (min)*	67 ± 2	59 ± 4	63 ± 8	67 ± 6

All values expressed as mean ± standard error of the mean.
*No significant differences among the four groups.

Statistics. Statistical analysis of the data was performed by analysis of variance and Scheffe's multiple comparison test. A p value less than 0.05 was considered significant. All data are expressed as mean (± standard error of the mean).

Animal care. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Results

Donor and recipient data. Flushing time, cold ischemic time, and warm ischemic time did not differ significantly among the four groups (Table II). The quality of flushing, according to subjective criteria such as rapid blanching and absence of mottling, was equivalent in the four groups. No significant differences were detected among the four groups with respect to arterial oxygen tension, peak inspiratory pressure, or PVR before harvesting or transplantation.

After left single-lung transplantation, the right pulmonary artery and right main bronchus were ligated 30 minutes after reperfusion; survival of the recipient animal thus depended solely on the left transplanted lung. All but two animals survived the final assessments. One animal in group III and one in group IV died after assessment, 3 hours after reperfusion.

Arterial blood gas analysis. The arterial oxygen tension of the transplanted lungs preserved with new ET-K in group I was uniformly excellent during the 6 hours of measurement and was significantly higher than that in group II with ET-K, group III with ET-KA, or group IV with LPDG (Fig. 1). Decreases in arterial oxygen tension were found starting 1 hour after reperfusion in groups II, III, and IV.

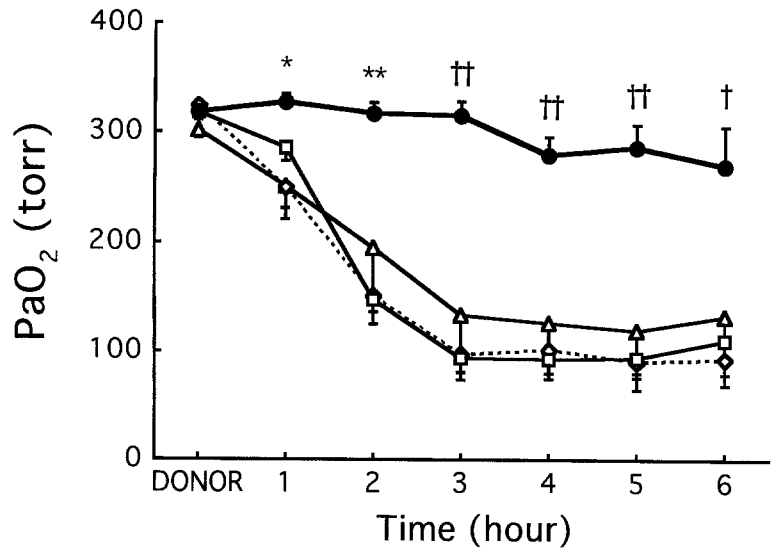

Fig. 1. Arterial oxygen tension (Pao_2) of transplanted lungs (inspired oxygen fraction 0.5). *Filled circles,* Group I (new ET-K); *open squares,* group II (ET-K); *open triangles,* group III (ET-KA); *open diamond,* group IV (LPDG). *Asterisk* indicates $p < 0.05$, group I versus group III or IV; *double asterisk* indicates $p < 0.01$, group I versus group II or IV, $p < 0.05$, group I versus group III; *dagger* indicates $p < 0.01$, group I versus group II, III, or IV; *double dagger* indicates $p < 0.05$, group I versus group II, III, or IV.

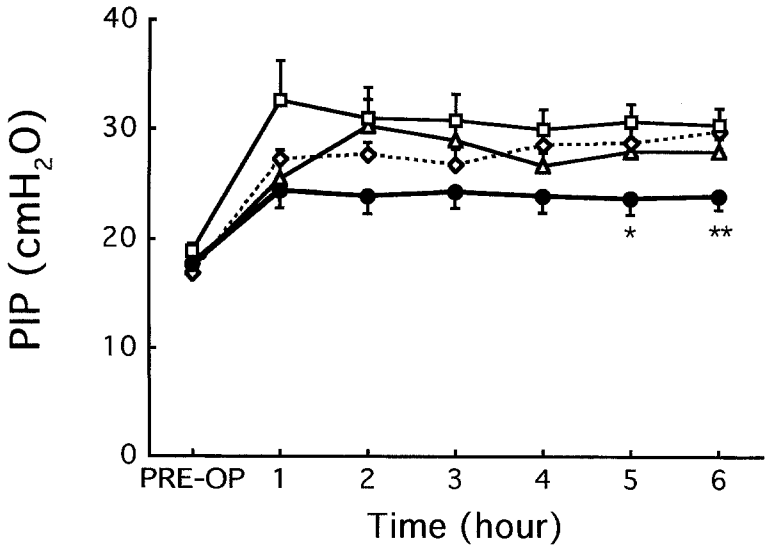

Fig. 2. Peak inspiratory pressure *(PIP)* of transplanted lungs. *Filled circles,* Group I (new ET-K); *open squares,* group II (ET-K); *open triangles,* group III (ET-KA); *open diamond,* group IV (LPDG); *PRE-OP,* before operation on recipients. *Asterisk* indicates $p < 0.05$, group I versus group II; *double asterisk* indicates $p < 0.01$, group I versus group II or IV.

Peak inspiratory pressure. The peak inspiratory pressure in group I was stable during the 6 hours of assessment and was significantly lower than that in group II or IV after 5 to 6 hours of reperfusion ($p < 0.05$ vs group II 5 hours after reperfusion, $p < 0.01$ vs group II or IV 6 hours after reperfusion; Fig. 2).

PVR. After 6 hours of reperfusion, the PVR of the transplanted lung was 2164 ± 260 dyne · sec · cm^{-5} in group I, 3057 ± 84 dyne · sec · cm^{-5} in group II,

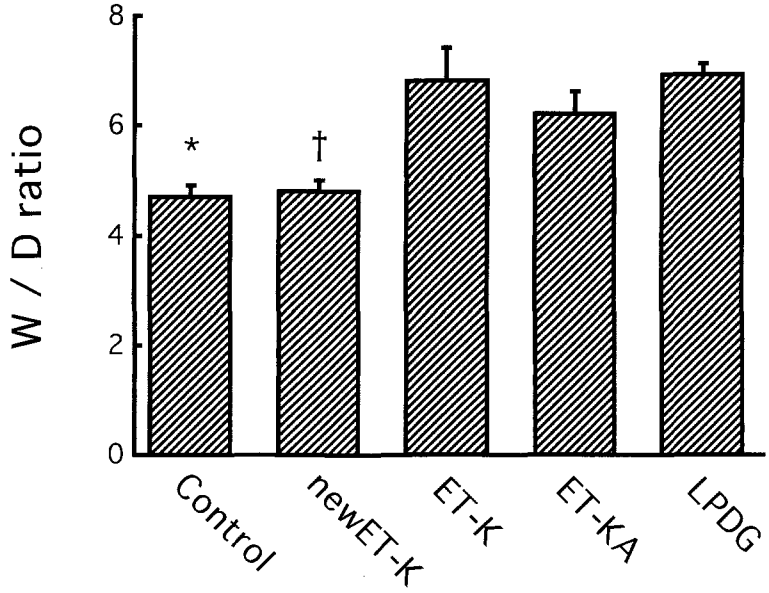

Fig. 3. Wet-to-dry *(W/D)* lung weight ratio of transplanted lungs. Asterisk indicates $p < 0.01$, control (resected native left lungs) versus group II (ET-K), III (ET-KA), or IV (LPDG). Dagger indicates $p < 0.01$ group I (new ET-K) versus group II or IV. No significant difference was observed between control group and group I or between groups I and III.

2454 ± 240 dyne · sec · cm^{-5} in group III, and 2739 ± 267 dyne · sec · cm^{-5} in group IV. There were no significant differences among the four groups.

Wet-to-dry lung weight ratio. The wet-to-dry lung weight ratios of transplanted lungs in each group are shown in Fig. 3. The wet-to-dry lung weight ratio of the transplanted lungs in group I was 4.8 ± 0.2, almost the same as that of the resected native left lungs (4.7 ± 0.2, $n = 6$). In contrast, the wet-to-dry lung weight ratios in the other three groups were significantly increased ($p < 0.01$ vs resected native left lungs). Significant differences were observed between group I and group II or group IV ($p < 0.01$).

Histologic findings. Histologic examination showed that the transplanted lungs in group I reperfused for 6 hours were essentially normal in structure, with no sign of pulmonary edema. In contrast, evidence of pulmonary edema (exudate accumulation in alveoli and thickening of the intraalveolar septa) was observed in all animals in groups II, III, and IV.

SEM examination. SEM study after 30 hours of cold storage revealed that donor lungs preserved with new ET-K (group I) showed essentially normal pulmonary arterial endothelial structures (Fig. 4, *A*). In contrast, significant pulmonary arterial endothelial cell swelling and protrusion were observed in group II (Fig. 4, *B*), group III (Fig. 4, *C*), and group IV (Fig. 4, *D*).

Discussion

In this study, the lungs preserved in new ET-K showed essentially normal ultrastructures in SEM examinations after 30 hours of cold storage. The transplanted lungs preserved in new ET-K functioned well.

In lung preservation and transplantation procedures, pulmonary vascular endothelial cells suffer heavy oxidant injury from extracellular and intracellular free oxygen radicals, which are produced in vascular endothelial cells and in leukocytes.[5, 14, 23] *N*-acetylcysteine is a powerful membrane-penetrating scavenger of reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid.[24] It reduces extracellular and intracellular radicals directly or by replenishment of cysteine and glutathione.[25] Sala and associates[6] reported that *N*-acetylcysteine protects pulmonary vascular endothelial cells against damage by oxygen free radical. The protective action of *N*-acetylcysteine against ischemia-reperfusion injury of transplanted lungs was reported in our previous study.[13] NO and cAMP are intercellular and intracellular second messengers with important roles

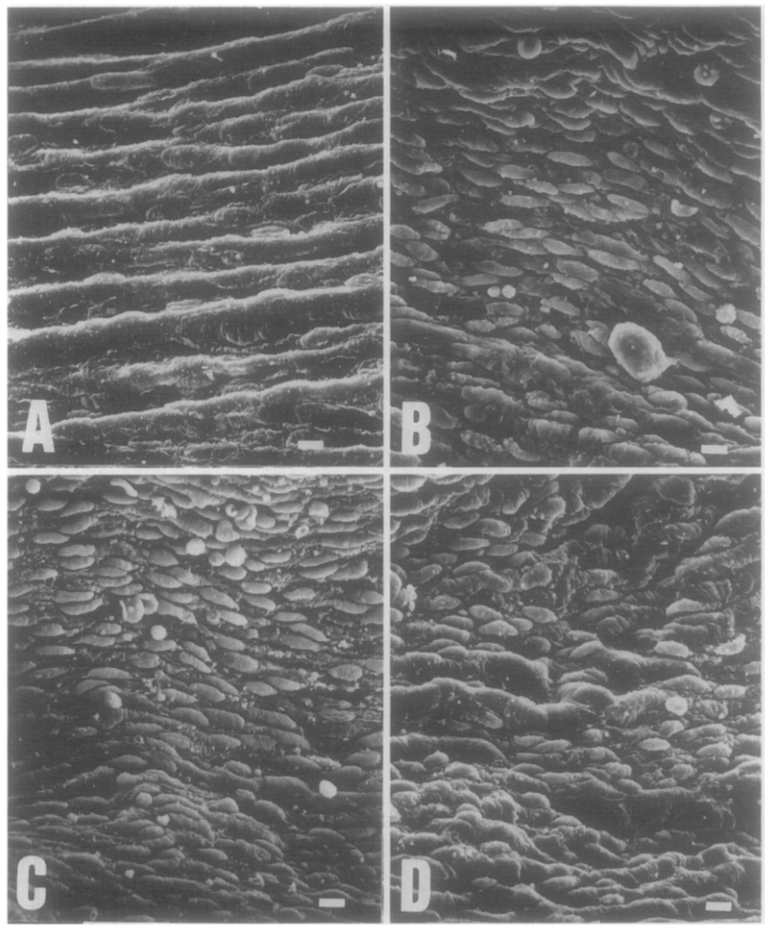

Fig. 4. Representative SEM photomicrographs of pulmonary arterial endothelium of preserved donor lungs. **A**, Essentially normal endothelial structures are seen in group I. Significant endothelial cell swelling and protrusion are seen in groups II (**B**), group III (**C**), and group IV (**D**). *Lines* indicate 5 μm.

in the control of vascular homeostasis and in preventing the production of oxygen free radicals in leukocytes.[8-12, 23, 26] During ischemia and reperfusion, both the NO and cAMP levels in vascular endothelial cells are reduced,[7, 8] leading to increased endothelial cell permeability, induced procoagulant activity, and leukocyte infiltration.[17, 27, 28] Nitroglycerin is a precursor of NO that replenishes NO in the grafts during ischemia and reperfusion. Naka and associates[15] reported that nitroglycerin supplementation in the preservation solution significantly improved the functions of transplanted lungs preserved in this solution. On the other hand, db-cAMP, a membrane-penetrating cAMP analog, raises the cAMP level either by being converted directly to cAMP or by inhibiting phosphodiesterase activity to prevent cAMP catabolism. Adkins and associates[16] reported that db-cAMP either prevents an increase in pulmonary vascular permeability caused by ischemia-reperfusion injury or inhibits the ability of neutrophils to damage endothelial cells through a mechanism involving an increase in intracellular levels of cAMP. In this study, lungs preserved in new ET-K, which contains *N*-acetylcysteine, nitroglycerin, and db-cAMP, had essentially normal vascular endothelial structures and histologic structures and had significantly improved oxygenation—better than those of lungs preserved in basal ET-K. These results suggest that the combination of these components is important in maintaining the integrity of the pulmonary vascular endothelium and potentiating pulmonary function.

Further studies are necessary to determine the effectiveness of each of these components in long-

term lung preservation. Oz and associates[17] reported that nitroglycerin and db-cAMP in the preservation solution were effective in cardiac storage, but they did not note any effect of *N*-acetylcysteine. Because their concentration of *N*-acetylcysteine was only a twentieth of that in our new ET-K, the effectiveness of *N*-acetylcysteine could not be ruled out in our study. It has been reported that nitroglycerin or db-cAMP prevents an increase in PVR after short-term lung ischemia.[15, 16] In this study, however, we did not observe significant differences in PVR of transplanted lungs preserved in the tested solutions; nitroglycerin and db-cAMP in new ET-K do not seem to cause vasodilation during relatively long-term lung preservation.

In this study, we compared new ET-K with ET-KA to which we added ethanol and propylene glycol in the same concentrations. Both solutions have higher osmolarity than that of basal ET-K. In this study, ET-KA did not improve lung preservation as well as did new ET-K; the lungs preserved in ET-KA showed poor pulmonary function and histologic and ultrastructural abnormalities similar to those preserved in basal ET-K. These findings indicate that ethanol and propylene glycol probably did not contribute to the excellent preservation efficacy of new ET-K; the hyperosmolarity of new ET-K appears to have little or not effect on its capacity for long-term preservation.

LPDG, the other solution tested, is an extracellular-type solution with a low potassium concentration. It has been reported that LPDG provides reliable 24-hour canine lung preservation.[21] In this study, lungs preserved in LPDG did not show better function than those preserved in new ET-K.

In conclusion, we demonstrated that our newly developed new ET-K, which contains *N*-acetylcysteine, nitroglycerin, and db-cAMP, enhances 30-hour lung preservation. The effects of the components in new ET-K need further study. These findings are expected to contribute to the improvement of clinical lung transplantation.

REFERENCES

1. Griffith BP, Zenati M. The pulmonary donor. Clin Chest Med 1990;11:217-26.
2. Cooper JD. Current status of lung transplantation. Transplant Proc 1991;23:2107-14.
3. Hardesty RL, Aeba R, Armitage JM, Kormos RL, Griffith BP. A clinical trial of University of Wisconsin solution for pulmonary preservation. J Thorac Cardiovasc Surg 1993;105: 660-6.
4. Kosaka S, Bando T, Liu CJ, Hitomi S, Wada H. Ultrastructural changes of pulmonary vasculature in canine lung preserved for 20 hours in newly developed solutions. Transplant Proc 1996;28:1841-2.
5. Paull DE, Keagy BA, Kron EJ, Wilcox BR. Improved lung preservation using a dimethylthiourea flush. J Surg Res 1989;46:333-8.
6. Sala R, Moriggi E, Corvasce G, Morelli D. Protection by *N*-acetylcysteine against pulmonary endothelial cell damage induced by oxidant injury. Eur Respir J 1993;6:440-6.
7. Pinsky DJ, Naka Y, Chowdhury NC, Liao H, Oz MC, Michler RE, et al. The nitric oxide/cyclic GMP pathway in organ transplantation: critical role in successful lung preservation. Proc Natl Acad Sci U S A 1994;91:12086-90.
8. Ogawa S, Koga S, Kuwabara K, Brett J, Morrow B, Morris SA, et al. Hypoxia-induced increased permeability of cultured bovine pulmonary and aortic endothelial monolayers occurs through lowering of cellular cAMP levels and is modulated by glucocorticoids. Am J Physiol 1992;262:C546-54.
9. Kubes P, Granger DN. Nitric oxide modulates microvascular permeability. Am J Physiol 1992;262(2 Pt 2):H611-5.
10. Kubes P, Suzuki M, Granger D. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci U S A 1991;88:4651-5.
11. Archipoff G, Beretz A, Froger-Leon, de la Salle C, Klein-Soyer C, Cazenave H. Opposite regulation by cAMP of thrombomodulin and tissue factor activities on the surface of human saphenous vein endothelial cells in culture [abstract]. Thromb Haemost 1991;65:349.
12. Boxer L, Allen J, Baehner R, Amick V. Diminished polymorphonuclear adherence. J Clin Invest 1980;66:268-74.
13. Yagi K, Liu CJ, Bando T, Yokomise H, Inui K, Hitomi S, et al. Inhibition of reperfusion injury by human thioredoxin (adult T-cell leukemia-derived factor) in canine lung transplantation. J Thorac Cardiovasc Surg 1994;108:913-21.
14. Lambert CJ, Egan TM. Optimal timing of administration of a free radical scavenger in lung preservation. Transplantation 1992;54:205-9.
15. Naka Y, Chowdhury NC, Oz MC, Smith CR, Yano OJ, Michler RE, et al. Nitroglycerin maintains graft vascular homeostasis and enhances preservation in an orthotopic rat lung transplant model. J Thorac Cardiovasc Surg 1995;109: 206-11.
16. Adkins WK, Barnard JW, May S, Seibert AF, Haynes J, Taylor AE. Compounds that increase cAMP prevent ischemia-reperfusion pulmonary capillary injury. J Appl Physiol 1992;72:492-7.
17. Oz MC, Pinsky DJ, Koga S, Liao H, Marboe CC, Han D, et al. Novel preservation solution permits 24-hour preservation in rat and baboon cardiac transplant models. Circulation 1993;88(5 Pt 2):II291-7.
18. Liu CJ, Bando T, Hirai T, Hirata T, Yagi K, Yokomise H, et al. Improved 20-hour canine lung preservation with a new solution-ET-Kyoto solution. Eur J Cardiothorac Surg 1995; 9:548-52.
19. Bando T, Kosaka S, Liu CJ, Hirai T, Hirata T, Yokomise H, et al. Effects of newly developed solutions containing trehalose on twenty-hour canine lung preservation. J Thorac Cardiovasc Surg 1994;108:92-8.
20. Keshavjee SH, Yamazaki F, Yokomise H, Cardoso PF, Mullen JB, Slutsky AS, et al. The role of dextran 40 and potassium in extended hypothermic lung preservation for transplantation. J Thorac Cardiovasc Surg 1992;103:314-25.

21. Date H, Matsumura A, Manchester JK, Obo H, Lima O, Cooper JM, et al. Evaluation of lung metabolism during successful twenty four–hour canine lung preservation. J Thorac Cardiovasc Surg 1993;105:480-91.
22. Hirata T, Fukuse T, Liu CJ, Muro K, Yokomise H, Yagi K, et al. Effects of trehalose in canine lung preservation. Surgery 1994;115:102-7.
23. Rubanyi GM, Ho EH, Cantor EH, Lumma WC, Parker-Botelho LH. Cytoprotective function of nitric oxide: inactivation of superoxide radicals produced by human leukocytes. Biochem Biophys Res Commun 1991;181: 1392-7.
24. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radical Biol Med 1989;6:593-7.
25. Butterworth M, Upshall DG, Hobbs M, Cohen GM. Elevation of cysteine and replenishment of glutathione in rat lung slices by cysteine isopropylester and other cysteine precursors. Biochem Pharmacol 1993;45:1769-74.
26. Bessler H, Gilgal R, Djaldetti M, Zahavi I. Effect of pentoxifylline on the phagocytic activity, cAMP levels, and superoxide anion production by monocytes and polymorphonuclear cells. J Leukocyte Biol 1986;40:747-54.
27. Pinsky DJ, Oz MC, Koga S, Taha Z, Broekman MJ, Marcus AJ, et al. Cardiac preservation is enhanced in a heterotopic rat transplant model by supplementing the nitric oxide pathway. J Clin Invest 1994;93:2291-7.
28. Pinsky D, Oz M, Liao H, Morris S, Brett J, Sciacca R, et al. Restoration of the cAMP second messenger pathway enhances cardiac preservation for transplantation in a heterotopic rat model. J Clin Invest 1993;92:2994-3002.